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YEAST FLORA OF RAW MILK IN EL-MINIA CITY, EGYPT

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ABSTRACT - 126 yeast strains were isolated from raw milk samples collected from milk sellers at Al-Minia city, upper Egypt. On the basis of 24 morphological and physiological merkmals, the isolated yeast strains were assigned to 13 genera. *Debaryomyces hansenii* and *Clavispora lusitaniae* were the most dominant species followed by *Trichosporon beigelii* and *Rhodotorula mucilaginosa*. *Kluyveromyces marxianus*, a lactose fermanting species, was also isolated. It is worthmentioning that the presence of the pathogenic species *Trichosporon beigelii* and *Rhodotorula mucilaginosa* constitutes a public health hazard and care should be taken for free milk from these human pathogens before being used.

RÉSUMÉ - A El-Minia, en Haute-Egypte, 126 souches de levures ont été isolées de lait frais, vendu par les commerçants de la ville. Sur la base des caractéristiques morphologiques et physiologiques, 24 espèces, réparties dans 13 genres, ont été répertoriées. Les plus répandues sont: *Debaryomyces hansenii* et *Clavispora lusitaniae*, suivies de *Trichosporon beigelii* et de *Rhodotorula mucilaginosa*. On dénombre également *Kluyveromyces marxianus* qui fermente le lactose. Il convient d'indiquer que la présence d'espèces nuisibles comme *Trichosporon beigelii* et *Rhodotorula mucilaginosa* représente un problème pour l'hygiène publique et nécessite la purification du lait avant sa consommation.

KEY WORDS : yeast, milk, human pathogens, *Trichosporon beigelii*, *Rhodotorula mucilaginosa*.

Raw milk represents an ideal growth medium for bacteria, mould and yeasts (Dombrowski, 1910). Although intensive studies were carried out on spoilage of milk with moulds and bacteria, few studies were performed on the role of yeasts in this process. Recently, microbiologists have focused their attention on yeasts as food spoilage causative organisms in raw milk and milk products such as cheese, yoghourt and butter (Davis, 1975; Busse, 1978; Kielwein, 1982; Spillmann & Ceiges, 1983). Along that line, yeasts were found to produce volatile acids (Hosono & Tokita, 1970; Kang et al., 1976; Engel, 1980; Winkelmann, 1989; Lenoir, 1984), hydrolyse proteins and fats (Schmidt et al., 1979; Alifax et al., 1982; Schmidt, 1982) and ferment lactose forming CO_2 which leads to swelling and blowing off the product container (Kiss et al., 1966; Davis, 1970; Kroger, 1976; Busse, 1978; Spillmann & Ceiges, 1983).

Although the occurrence of yeasts in raw milk was reported by some investigators in several countries (Bridge Cooke & Brazis, 1968; Nakanishi & Arai, 1968, 1969; Rey Fernandez, 1972; Deiana et al., 1977; Haridy, 1987), few investigations were carried out along that line in Egypt and were mainly concerned with cheese and butter (Fahmy & Youssef, 1974; El-Gendy et al., 1979; El-Bassiony et al., 1980; Se-

ham et al., 1982; Zein et al., 1983), whereas none was performed on yeast flora of raw milk. In this paper we report on the yeast flora of raw milk samples collected from El-Minia city in upper Egypt in an attempt to evaluate the role of yeasts in spoilage of raw milk in this locality.

MATERIALS AND METHODS

Raw milk samples were collected from milk sellers in sterile test tubes and were directly transported to the laboratory. Sampling was performed 2-3 times every month for a period of one year starting January 1990 to January 1991. For isolation and identification of yeast strains, 0.2 ml portions of raw milk were aseptically withdrawn and directly spread on YM-agar plates adjusted to pH 3.5 (Lodder, 1970) or mixed in a test tube with 0.8 ml sterile distilled water and decimal dilutions were prepared for counting purposes. Inoculated plates were inoculated at 28°C for 2-3 days. Developing yeast colonies were counted and after microscopic examination, strains were isolated, purified and preserved on YM-agar slants at 4°C. Identification of yeast strains was performed according to the standard keys of yeast identification (Lodder, 1970; Barnett et al., 1983; Kreger van Rij, 1984).

RESULTS AND DISCUSSION

Table 1 shows the total yeast count of raw milk at different months during the sampling period and the level of occurrence of isolated species. All the tested samples contained yeast cells with a total count ranging between 1.8×10^2 and 2×10^4 cells per 1 ml raw milk. The highest count of yeast cells was recorded in April, May and September while the lowest number of yeast cells was found in December. In Italy, yeast count of milk was 2×10^3 ml^{-1} (Fatichenti et al., 1977).

Table 2 shows that a total of 126 yeast strains were isolated from milk samples and identified to the species level and these were found to belong to 13 genera which are: *Debaryomyces* (27 isolates), *Clavispora* (24 isolates), *Trichosporon* (22 isolates), *Rhodotorula* (16 isolates), *Kluyveromyces* (10 isolates), *Torulaspora* (8 isolates), *Cryptococcus* (6 isolates), *Hansenula* (4 isolates), *Saccharomyces* (4 isolates) and *Candida* (2 isolates). Each of the genera *Dekkera*, *Schwanniomyces* and *Sporidiobolus* were represented by a single strain. *Debaryomyces hansenii* and *Clavispora lusitaniae* were more frequently isolated and occurred in high numbers of cells (table 1). *Trichosporon beigelii* and *Rhodotorula mucilaginosa* were regularly isolated but with low numbers of cells. Data indicated that these four species were capable of substantial growth in raw milk. These species were isolated by Haridy (1987) from raw milk in west Germany and the species *Debaryomyces hansenii* was the most dominant.

Kluyveromyces marxianus, a lactose fermenting species, was also isolated and was represented by 7.9% of the total isolates. The occurrence of this species in raw milk is previously reported by several investigators (Ingram, 1958; Nakanishi & Arai, 1968, 1969; Walker & Ayres, 1970; Peppler, 1976; Haridy, 1987).

The remaining yeast species listed in table 1 were sporadically isolated with few cell numbers and therefore were considered as contaminants that were unable to be established in raw milk.

Data in table 2 shows that, fermentation of glucose, assimilation of lactose and building of ascospores and true mycelium represented the differential merkmals for *Debaryomyces hansenii*, *Clavispora lusitaniae*, *Trichosporon beigelii* and *Rhodotorula*

Table I: Distribution of yeast species in raw milk during 12 months period.
Tableau I - Répartition des levures dans le lait frais pendant 12 mois.

	January	Februa.	March	April	May	June	July	August	Septemb.	October	Novemb.	December
Total count of yeast cells/ml raw milk	$2.0x10^3$	$1.1x10^3$	$1.7x10^3$	$1.3x10^4$	$2.0x10^4$	$1.0x10^3$	$1.4x10^3$	$1.3x10^3$	$2.0x10^3$	$1.2x10^3$	$1.0x10^1$	$1.8x10^2$
Debaryomyces hansenii	+++	+++	++	+++	++	++	++	++	+	+	+++	+++
Clavispora lusitaniae	+	+++	+++	+++	++	++	++	+	+++	+++	+++	+
Trichosporon beigelii	+	+	+	+	+	+	+	+	+	+	+	+
Rhodotorula mucilaginosa	++	+	+	+	+	+	+	+	+	+	+	++
Kluyveromyces marxianus	+	+	+++						+	+		
Torulaspora delbrueckii		+	+++	+								
Cryptococcus laurentii		+									+	+++
Hansenula polymorpha										+	+++	++
Saccharomyces cerevisiae	++			+++								
Dekkera intermedia	+++											
Candida blankii	+											
Candida apis												
Sporidiobolus salmonicolor			+									
Debaryomyces vanrijii				+								
Schwanniomyces occidentalis												+

+++ = high number of colonies, ++ = moderate number of colonies, + = few number of colonies

Table 2 - Physiological and morphological properties of the isolated yeast species (*)
Tableau 2 - Propriétés physiologiques et morphologiques des levures isolées.

SPECIES	number of strains tested	FERMENTATION					ASSIMILATION											GROWTH AT		BUILDING OF			
		glucose	galactose	maltose	sucrose	lactose	galactose	sorbose	ribose	xylose	arabinose	rhamnose	sucrose	maltose	lactose	raffinose	mannitol	succinate	citrate	37°C	42°C	ascospores	pseudomycelium	true mycelium	pellicle
Debaryomyces hansenii	26	100	62	54	54	0	100	85	46	100	50	62	100	100	23	100	100	100	100	54	0	100	50	0	54
Clavispora lusitaniae	24	100	100	42	75	0	100	100	42	100	0	100	100	100	■	100	100	100	17	100	84	100	100	0	100
Trichosporon beigelii	22	0	0	■	0	0	100	64	73	100	100	55	100	91	100	100	82	91	82	64	27	■	100	100	100
Rhodotorula mucilaginosa	16	0	0	0	0	0	100	88	100	88	38	38	100	100	0	100	100	100	25	63	0	0	0	0	0
Kluyveromyces marxianus	10	100	100	20	100	100	100	100	20	60	60	0	100	80	100	100	80	100	40	40	20	100	100	0	100
Torulaspora delbrueckii	■	100	0	75	25	0	50	25	0	100	0	0	100	100	0	100	0	25	0	75	■	100	50	■	50
Cryptococcus laurentii	6	■	0	0	0	0	100	100	100	100	100	67	100	100	100	100	100	100	100	100	0	0	0	0	0
Hansenula polymorpha	4	100	■	0	50	0	50	100	50	50	50	0	100	100	0	0	100	0	0	100	100	100	0	0	0
Saccharomyces cerevisiae	4	100	100	0	0	0	100	0	0	0	0	0	100	50	0	50	100	50	0	50	50	100	100	■	100
Debaryomyces vanrij	1	0	0	0	0	0	100	100	■	0	0	0	100	0	0	100	100	■	0	100	■	100	0	0	0
Dekkera intermedia	1	100	0	0	0	0	0	0	0	0	0	0	100	100	0	100	0	0	0	100	0	100	100	0	100
Schwanniomyces occidentalis	■	100	100	0	0	0	100	100	100	100	0	0	100	100	0	100	100	100	100	0	0	100	0	0	0
Candida apis	1	0	0	0	0	0	100	100	0	100	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Candida blankii	1	100	100	100	100	0	100	100	100	100	0	100	100	100	100	100	100	100	100	100	100	0	100	100	100
Sporidiobolus salmonicolor	1	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100

* percentages of positive reactions of strains

mucilaginosa which are the most dominant species. Building of arthrospores and asexual reproduction of fission were additive characteristics for *Trichosporon beigelii*. Building of pigmented colonies, which are shiny, pink or red with entire margin, was of value in case of *Rhodotorula mucilaginosa*.

Of particular interest was the isolation of *Trichosporon beigelii* and *Rhodotorula mucilaginosa* in considerable numbers. These two species were reported as human pathogens by Hurley et al. (1987). Their presence in raw milk may be deleterious to human health and care should be taken to insure that raw milk should be free from them before being used.

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